Procedures for the Manual Extraction of DNA

1 Scope

These procedures apply to DNA personnel performing manual extraction of deoxyribonucleic acid (DNA) using Phenol/Chloroform/Isoamyl Alcohol (PCIA) and Microcon® filters in the DNA Casework Unit (DCU) or Scientific and Biometrics Analysis Unit (SBAU).

2 Equipment/Materials/Reagents

Equipment/Materials

- General laboratory supplies (e.g., tubes, pipettes, vortex, centrifuge)
- Incubator (Thermo MaxQ 4450 or 4000, Thermo 6841, Labline Imperial III, Heratherm IGS 100, or equivalent) or thermomixer (Eppendorf Thermomixer 5350s or equivalent)
- Qiagen® Lyse & Spin Baskets and Collection Tubes, or equivalent (***NOT** for differential extractions*)
- Costar® spin baskets, or equivalent (*for differential or normal extractions*)
- Phase Lock Tubes, 1.5 mL or 2 mL (Phase Lock Gel ™ Low or High Density Gel, Qiagen® MaXtract High Density, or equivalent)
- Microcon® DNA Fast Flow Centrifugal Filter Device and Tubes (EMD Millipore Corporation or equivalent)

Reagents

- 25:24:1 Phenol/Chloroform/Isoamyl Alcohol (PCIA)
- Proteinase K, 20 mg/ml
- Water, Reagent Grade or equivalent
- Stain Extraction Buffer (SEB) with Dithiothreitol (DTT) (*for normal extractions*)
- TNE Buffer (TNE) (*for differential extractions*)
- Sarkosyl, 20 mg/ml (*for differential extractions*)
- Sperm Wash Buffer (*for differential extractions*)
- Dithiothreitol (DTT), 1M (*for differential extractions*)

Refer to the appropriate DNA procedure (i.e., DNAQA 609) for reagent preparation information.

3 Standards and Controls

At least one extraction control (i.e., reagent blank) must be processed in parallel with each extraction batch. The reagent blank(s) will be processed as the last sample(s) in the batch.

For evaluation of the extraction controls, refer to the appropriate interpretation procedure of the *DNA Procedures Manual* (i.e., DNA 233, DNA 215, DNA 410).

4 Procedures

Refer to the DNA Procedures Introduction (i.e., DNAQA 600) for applicable general precautions and cleaning instructions.

Ensure the appropriate fields (i.e., instruments, reagents) in STACS are completed, as necessary.

4.1 Normal Extraction Lysis

Lyse & Spin baskets in corresponding tubes are typically used for normal extractions when using an incubator. If using a thermomixer, the Lyse & Spin baskets should not be used.

4.1.1	Create master mix for the extraction batch.	

Normal Extraction Master Mix

Reagent	µL per sample
SEB w/DTT	450
Pro K	3

4.1.2	Add 450 µL master mix.	

4.1.3	Vortex, quick spin and incubate with agitation at 56ºC for 2-4 hours.	

4.1.4	If necessary, quick spin and transfer the cutting to an appropriate basket. Spin tubes (generally between 9,000 and 13,000 rpm for 5 minutes), discard basket.	

Proceed to PCIA and Microcon Purification, Section 4.3.

4.2 Differential Extraction Lysis and Fractionation

Lyse & Spin baskets must NOT be used for differential extractions.

4.2.1	Create master mix for the extraction batch.	

Differential Extraction Master Mix

Reagent	µL per sample
TNE	400
Sarkosyl	25
Reagent grade water	75
Pro K	1

4.2.2	Add 450 µL master mix.	
4.2.3	Vortex, quick spin and incubate with agitation at 37ºC for 2-4 hours.	
4.2.4	Quick spin. If necessary, transfer cutting to an appropriate basket, spin (generally between 9,000 and 13,000 rpm for 5 minutes), discard basket.	
4.2.5	Avoiding the pellet, transfer the supernatant into a new labeled microcentrifuge tube.	

The supernatant is the epithelial (F) fraction. The cell pellet remaining in the tube is the sperm (M) fraction. Processing of the F fraction resumes at PCIA and Microcon Purification, either independently or with the M fraction.

4.2.6 Sperm Wash

4.2.6.1	Add 450 µL Sperm Wash Buffer to the M fraction tubes.	
4.2.6.2	Vortex and spin (generally between 9,000 and 13,000 rpm for 5 minutes).	
4.2.6.3	Remove and discard the supernatant, avoiding the pellet.	
4.2.6.4	Repeat sperm wash steps two additional times.	

4.2.7 Male Fraction Lysis

4.2.7.1	Ensure the M fraction master mix has been created for the extraction batch.	

M Fraction Master Mix

Reagent	µL per sample
TNE	225
Sarkosyl	75
Reagent grade water	225
DTT	10.5
Pro K	3

4.2.7.2	Add 450 µL M fraction master mix to the M fraction tubes.	
4.2.7.4	Vortex, quick spin and incubate with agitation at 37ºC for 2-4 hours.	

Proceed to PCIA and Microcon Purification.

4.3 PCIA and Microcon Purification

The PCIA should be allowed to equilibrate to room temperature prior to use.

If differential extracts are processed simultaneously, the M fractions and their corresponding reagent blanks are processed through each step of the purification prior to the F fractions and their corresponding reagent blanks.

4.3.1	If needed, quick spin all tubes. In a fume hood, add 450 µL PCIA to each tube.	

Dispose of PCIA and all consumables (i.e., tips, tubes) that come into contact with PCIA in an appropriate waste container.

4.3.2	When using phase lock tubes: Spin the phase lock tubes (generally between 9,000 and 13,000 rpm for 30 seconds) to pellet the phase lock gel. Vortex, quick spin and add entire volume of PCIA/lysate emulsion to pelleted, labeled phase lock tube.	

4.3.3	Spin tubes (generally between 9,000 and 13,000 rpm for 5 minutes).	

4.3.4	Transfer top layer to a labeled microcon assembly. Appropriately discard the tube containing the bottom layer.	

NOTE: If phase lock tubes are not used, the upper aqueous layer is transferred to the microcon assembly taking care not to pipette the bottom layer or the interface between the layers.

Steps 4.3.4 through 4.3.6 will be repeated, as necessary, for those extracts and corresponding reagent blanks being combined. If multiple extracts are to be combined (e.g., multiple swabs or cuttings extracted separately, to include those previously extracted), add only one extract to the microcon at a time and spin prior to adding subsequent extract to the microcon.

4.3.5	Spin the labeled microcon assemblies (generally between 6,000 and 8,000 rpm for 10 minutes).	

Additional spins may be used to draw fluid through the membrane. Speed and/or time may be increased, but excess speed and/or time should be avoided to prevent damaging the membrane.

4.3.6	Discard waste. (*By decanting or pipetting, entire waste volume does not need to be removed*.)	

4.3.7	Add 200 µL reagent grade water to each microcon.	

4.3.8	Spin (generally between 6,000 and 8,000 rpm for 10 minutes).	

Additional spins may be used to draw fluid through the membrane. Speed and/or time may be increased, but excess speed and/or time should be avoided to prevent damaging the membrane.

If needed, additional reagent grade water washes (steps 4.3.6 through 4.3.8) may be performed and must be carried out in parallel on the corresponding reagent blank(s).

If additional spins or washes do not reduce the volume, the affected sample(s) may continue with processing at step 4.3.9. Record the final volume.

4.3.9	Add reagent grade water (generally 15 µL) to each microcon. Invert each microcon into a new, labeled microcon tube. Spin (generally between 9,000 and 13,000 rpm for 5 minutes).	

4.3.10	Ensure extracts are transferred to a robot compatible tube, if appropriate, and the final tubes are barcoded.	

If the final extract displays discoloration, a dilution may be prepared with reagent grade water.

Refer to the applicable DNA procedure (i.e., DNA 226 or DNA 232) if samples need to be further combined or concentrated following extraction.

6 Sampling

Not applicable.

7 Calculations

Not applicable.

8 Measurement Uncertainty

Not applicable.

9 Limitations

9.1 The quantity and quality of the DNA present within any biological material ultimately determines if a DNA extraction is successful.

10 Safety

10.1 All evidence containing or contaminated with blood or other potentially infectious materials will be considered infectious regardless of the perceived status of the source individual or the age of the material. Follow the "Safe Work Practices and Procedures," "Bloodborne Pathogen (BBP) Exposure Control Plan (ECP)," "Personal Protective Equipment Policy," and "Chemical Hygiene Plan" sections of the *FBI Laboratory Safety Manual*.

10.2 Refer to the "Hazardous Waste Disposal" section of the *FBI Laboratory Safety Manual* for important information concerning proper disposal of the chemicals used in these procedures as well as the biohazardous wastes generated.

10.3 Procedural Specific Chemical Hazards:

- Solutions of Proteinase K can be irritating to mucous membranes. Use eye protection when handling.
- PCIA (Phenol/Chloroform/Isoamyl Alcohol) can cause burns and is toxic by inhalation, contact with skin, and if swallowed. Its use will be confined to a fume hood.

11 References

FBI Laboratory Quality Assurance Manual

FBI Laboratory Operations Manual

FBI Laboratory Safety Manual

DNA Procedures Manual

5 Prime *Manual Phase Lock Gel™ (PLG) User Guide*, 2007.

Comey CT, Koons BW, Presley KW, Smerick JB, Sobieralski CA, Stanley DM, and Baechtel FS. DNA extraction strategies for amplified fragment length polymorphism analysis. *Journal of Forensic Sciences* (1994) 39: 1254-1269.

Millipore Corporation. *Microcon® Centrifugal Filter Devices User Guide.* Millipore Corporation, Billerica, MA, 2013. (Available at http://www.emdmillipore.com/Web-US-Site/en_CA/-/USD/ShowDocument-File?DocumentId=201306.3829.ProNet&ProductSKU=MM_NF-MRCF0R100&Language=EN&DocumentType=UG&Origin=PDP&Country=NF)

Qiagen® *MaXtract Low and High Density Handbook*, October 2006.

Rev. #	Issue Date	History
2	02/28/18	Adjusted scope to apply to DNA personnel. Relocated reagent lists to section 2. Updated DNA procedure references throughout. Changed all PCI abbreviations to PCIA. Consolidated PCIA and Microcon procedures to 4.3. Renamed prior sections.
3	06/01/21	Editorial modifications throughout. 1: Updated to SBAU 2: Added incubator/thermomixer. Clarified L&S baskets are not for diffs whereas Costar may be used for normals. 3: Added instruction for RB to be the last sample of a batch. Added interpretation SOP reference numbers. 4: Removed detail from any network computer. 4.1/4.2: Added L&S basket guidance 4.2.6: Added sperm wash header, removed from 4.2 header. 4.2.7: Added male lysis header. Renumbered sections. 4.3: Added corresponding RBs 4.3.2: Step only done when using phaselock tubes 4.3.4: Added detail to the note and reordered combining paragraph. 4.3.9: Clarified microcon tube 4.3.10: Added transferring to robot compatible tube, if appropriate.

Approval Redact - Signatures on File

DNA Technical Leader Date: 06/01/2021

DCU Chief Date: 06/01/2021

SBAU Chief Date: 06/01/2021